Immune Loss as a Driver of Coexistence During Host-Phage Coevolution

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Abstract

Bacteria and their viral pathogens face constant pressure for augmented immune and infective capabilities, respectively. Under this reciprocally imposed selective regime, we expect to see a runaway evolutionary arms race, ultimately leading to the extinction of one species. Despite this prediction, in many systems host and pathogen coexist with minimal coevolution even when well-mixed. Previous work explained this puzzling phenomenon by invoking fitness tradeoffs, which can diminish an arms race dynamic. Here we propose that the regular loss of immunity by the bacterial host can also produce host-phage coexistence. We pair a general model of immunity with an experimental and theoretical case study of the CRISPR-Cas immune system to contrast the behavior of tradeoff and loss mechanisms in well-mixed systems. We find that, while both mechanisms can produce stable coexistence, only immune loss does so robustly within realistic parameter ranges.

1 Introduction

While the abundance of bacteria observed globally is impressive (Hug *et al.*, 2016; Schloss *et al.*, 2016; Whitman *et al.*, 1998), any apparent microbial dominance is rivaled by the ubiquity, diversity, and abundance of predatory bacteriophages, which target

these microbes (Suttle, 2005; Weitz and Wilhelm, 2012; Wigington *et al.*, 2016; Wilhelm and Suttle, 1999; Wommack and Colwell, 2000). As one might expect, "phages" are powerful modulators of microbial population and evolutionary dynamics, and of the global nutrient cycles these microbes control (Bergh *et al.*, 1989; Bratbak *et al.*, 1990, 1994; Proctor and Fuhrman, 1990; Sieburth *et al.*, 1988; Suttle, 2005; Weinbauer and Rassoulzadegan, 2004; Weitz and Wilhelm, 2012; Whitman *et al.*, 1998; Wilhelm and Suttle, 1999). Despite this ecological importance, we lack a comprehensive understanding of the the dynamical behavior of phage populations. More specifically, it is an open question what processes sustain phages in the long term across habitats.

Phages coexist with their hosts in a variety of natural environments (e.g. Gómez and Buckling, 2011; Waterbury and Valois, 1993) and artificial laboratory systems (e.g. Bohannan and Lenski, 1999; Chao *et al.*, 1977; Horne, 1970; Lenski and Levin, 1985; Levin and Udovic, 1977; Páez-Espino *et al.*, 2015; Schrag and Mittler, 1996; Wei *et al.*, 2011) despite the fact that bacteria can evade phages using both passive forms of resistance (e.g. receptor loss, modification, and masking) and active immune systems that degrade phages (e.g. restriction-modification systems, CRISPR-Cas). These defenses can incite an escalating arms race dynamic in which host and pathogen each drive the evolution of the other (Rodin and Ratner, 1983a,b). However, basic theory predicts that such an unrestricted arms race will generally be unstable and sensitive to initial conditions (Schrag and Mittler, 1996).

These examples of apparently stable coexistence have motivated a search for mechanisms that might explain the deescalation and eventual cessation of a coevolutionary arms race dynamic, even in the absence of any spatial structure to the environment. Previous authors have identified (1) fluctuating selection and (2) costs of defense as potential drivers of coexistence in well-mixed systems. Here we propose (3) the loss of immunity as an additional mechanism. We focus on intracellular *immunity* (e.g., CRISPR-Cas) in which immune host act as a sink for phages rather than extracellular *resistance* (e.g., receptor modifications), since the former poses more of an obstacle for phages and thus more of a puzzle for explaining long-term coexistence.

Under a fluctuating selection dynamic, the frequencies of immune and infective alleles in the respective host and phage populations cycle over time (Agrawal and Lively, 2002; Gandon *et al.*, 2008; Van Valen, 1973, 1974). Host and phage almost certainly

cannot escalate an arms race indefinitely via novel mutations (Hall *et al.*, 2011; Lenski, 1984; Lenski and Levin, 1985), and the reemergence of old, rare genotypes provides a feasible alternative to the generation of new mutations. There is empirical evidence that escalating arms races give way to fluctuating selection dynamics in some host-phage systems (Hall *et al.*, 2011). In contrast to this result, when novel immune or infective phenotypes correspond to increased generalism we do not expect past phenotypes to recur (Agrawal and Lively, 2002; Gandon *et al.*, 2008) since they will no longer be adaptive. The expansion of generalism during coevolution has been shown to be typical of many experimental microbial systems (Buckling and Rainey, 2002). Thus we might expect a clear "winner" of the arms race in these situations, leading to a breakdown in coexistence. Therefore, while a fluctuating selection dynamic may explain long term coexistence in some systems, it does not seem to be sufficient to explain the majority of host-phage coexistence observed in the laboratory.

Another possible driver of coexistence are costs incurred by tradeoffs between growth and immunity (for host) or host range and immune evasion (for phage) (Chao *et al.*, 1977; Jover *et al.*, 2013; Levin *et al.*, 1977; Meyer *et al.*, 2016). If phage evolution is limited either due to architectural constraints or pleiotropic costs, then the bacterial host does not face pressure to continually evolve heightened defense. In this case, a tradeoff between increased immunity and growth rate in the host can lead to the maintenance of a susceptible host population on which phages can persist (Chao *et al.*, 1977; Jones and Ellner, 2007; Jover *et al.*, 2013; Lenski and Levin, 1985; Levin and Udovic, 1977; Yoshida *et al.*, 2007). Tradeoff-based mechanisms may drive coexistence in some systems, but they require a high cost of immunity that does not always apply (e.g. Schrag and Mittler, 1996). As we show later, when phages experience adsorption-dependent death from host immunity, their existence is relatively precarious and the cost of immunity must be extraordinarily high to result in coexistence.

Finally, in large host populations typical of bacteria, even a low rate of loss of immunity could produce a substantial susceptible host subpopulation, which, in turn, could support phage reproduction and coexistence. Delbrück (1946) initially described this hypothesis of loss of defense via back-mutation in order to challenge the evidence for lysogeny, differentiating between "true" and "apparent" lysogenesis. Lenski (1988) reiterated this hypothesis in terms of phenotypic plasticity and noted that conditioning

the production of a susceptible host population on a resistant one could lead to very robust, host-dominated coexistence. More recently, Meyer *et al.* (2012) presented an empirical example of a system in which stochastic phenotypic loss of resistance leads to persistence of a coevolving phage population even given an almost entirely resistant host population.

We hypothesize that coexistence equilibria will be more robust under an immune loss mechanism than under a tradeoff mechanism because the former conditions the production of susceptible host on a stable resource-limited immune host population. We build a general mathematical model to demonstrate this point and then use a combination of experimental evidence and simulation-based modeling to apply this result to the coevolution of *Streptococcus thermophilus* and its lytic phage 2972 in the context of CRISPR immunity.

2 General Immune Loss Model

We begin with a general model that considers two populations of host ("defended" with a functional immune system; "undefended" without) and one population of pathogen. Starting from previous work examining cryptic rotifer-algal dynamics (Jones and Ellner, 2007) and classic bacteria-phage dynamics (Levin *et al.*, 1977; Weitz, 2016), we add key terms to capture the effects of coevolution implicitly, autoimmunity (i.e., a tradeoff), and immune loss. This relatively simple model allows us to analyze steady states and parameter interactions analytically. Later, we examine the CRISPR immune system in detail and build a more complex model with explicit coevolutionary dynamics.

We examine the chemostat system with resources:

$$\dot{R} = w(A - R) - \frac{evR}{z + R}(D + U) \tag{1}$$

defended host:

$$\dot{D} = D\left(\frac{vR}{z + R} - \delta\phi_d P - \alpha - \mu - w\right), \tag{2}$$

undefended host:

$$\dot{U} = U\left(\frac{vR}{z + R} - \delta\phi_u P - w\right) + \mu D, \tag{3}$$

and phage:

$$\dot{P} = P\left(\delta U(\phi_u\beta - 1) + \delta D(\phi_d\beta - 1) - w\right), \quad (4)$$

where parameter definitions and values can be found in Table 1 and rationale/references for parameter values in S2 Text. However, we describe here the parameters of direct relevance to coexistence.

First, we model the effect of coevolution by allowing a fraction of even the defended host population to remain susceptible ($0 < \phi_d \leq 1$). In a symmetric fashion, even nominally undefended host may be partially resistant ($0 < \phi_u \leq 1$) due to secondary forms of defense, such as a restriction-modification system (Dussoix and Arber, 1962; Luria and Human, 1952).

Second, we allow for defended host to come with the tradeoff of autoimmunity (α), which applies naturally to the CRISPR system examined later. While autoimmunity could either decrease the host growth rate (Vercoe *et al.*, 2013) or be lethal, we focus on the latter as lethality will increase the stabilizing effect of this tradeoff (Dy *et al.*, 2013; Paez-Espino *et al.*, 2013; Vercoe *et al.*, 2013). However, we also find similar general results when applying a penalty to the resource affinity or maximum growth rate of the defended host (S1 Text, S1 Fig-S8 Fig).

Finally, we add flow from the defended to undefended host populations representing loss of immunity at rate μ.

We analyze our model analytically as well as numerically to verify that any equilibria are reachable from plausible (e.g., experimental) starting values (S3 Text).

Assuming no phage coevolution ($\phi_d = 0$), this model has a single analytic equilibrium in which all populations coexist (S1 Table). In Fig 1, we explore model behavior under varying rates of autoimmunity (α) and immune loss (μ). Clearly when autoimmunity and loss rates surpass unity, defended host go extinct in the face of excessive immune loss and autoimmune targeting. At the opposite parameter extreme, we see coexistence disappear from the numeric solutions (Fig 1b) as phage populations collapse. This leads to a band of parameter space where coexistence is possible, stable, and robust. In this band, autoimmunity and/or immune loss occur at high enough rates to ensure maintenance of coexistence, but not so high as to place an excessive cost on immunity. Crucially, this band is much more constrained in the α-dimension, with

autoimmunity restricted to an implausibly high and narrow region of parameter space. This suggests a greater robustness of coexistence under an immune loss mechanism even at low loss rates (Fig 1, S2 Fig-S9 Fig).

If we add large amounts of innate immunity to the system by decreasing ϕ_u, we find phage-dominated coexistence for a wider range of α (S11 Fig). This result is in line with the counterintuitive suggestion that higher immunity may increase phage density by allowing the host population to increase in size (Iranzo *et al.*, 2013). However, our model requires innate immunity with over a 50% effectiveness in combating phage infection to see even a small shift in behavior, suggesting that secondary defense in the "undefended" strain (e.g. innate envelope resistance) has minimal effects unless it provides near-complete protection.

In the case of phage coevolution ($\phi_d > 0$), the equilibria still have closed forms, but are not easily representable as simple equations and so are not written here. When $\phi_d > \frac{1}{\beta}$, defended host begin to contribute positively to phage growth, which leads to an eventual shift in the coexistence equilibrium from host to phage dominance (S10 Fig).

3 A Case Study: CRISPR-Phage Coevolution

The CRISPR (Clustered Regularly Inter-spaced Short Palindromic Repeats) prokaryotic adaptive immune system incorporates specific immune memory in the form of short sequences of DNA acquired from foreign genetic elements ("spacers") and then uses this memory to target the corresponding sequences ("protospacers") during subsequent infections (Barrangou *et al.*, 2007; Bolotin *et al.*, 2005; Garneau *et al.*, 2010; Mojica *et al.*, 2005). This system can lead to an arms race between bacteria and phage (Deveau *et al.*, 2008; Horvath *et al.*, 2008; Páez-Espino *et al.*, 2015) in which, in contrast to typical coevolutionary arms races, evolutionary dynamics occur on the same timescale as population dynamics (Childs *et al.*, 2012; Desai *et al.*, 2007; Gerrish and Lenski, 1998; Páez-Espino *et al.*, 2015).

How phages can persist in the face of this extremely adaptable immune system remains unclear. Previous theoretical and limited experimental work has explained short-term coexistence through tradeoffs and spacer loss (Bradde *et al.*, 2017) and extended bacteria-phage coexistence by invoking an arms race via negative

frequency-dependent selection (Childs *et al.*, 2012) or tradeoffs with host switching to a constitutive defense strategy such as surface receptor modification (Chabas *et al.*, 2016; Westra *et al.*, 2015).

However, these previous hypotheses are insufficient to explain simple coevolution experiments with *Streptococcus thermophilus* (type II-A CRISPR system) and its lytic phage 2972 resulting in long-term coexistence (Páez-Espino *et al.*, 2015). In these experiments, the bacterial density suggests limitation by resource rather than phage, which implies any arms race has ended and that phages are persisting on a susceptible subpopulation of host. Additionally, since experiments are carried out in liquid culture with daily serial dilutions, we do not expect spatial heterogeneity to play a role. These data thus imply that either (1) costs associated with CRISPR immunity or (2) the loss of CRISPR immunity is playing a role in maintaining susceptible host subpopulations on which phages can persist.

In this system, the primary cost of a functional CRISPR system is autoimmunity via the acquisition of self-targeting spacers. In general, it is unclear how or if bacteria distinguish self from non-self during the acquisition step of CRISPR immunity (Kumar *et al.*, 2015; Levy *et al.*, 2015; Stern *et al.*, 2010; Wei *et al.*, 2015; Yosef *et al.*, 2012). In *S. thermophilus*, experimental evidence suggests that there is no mechanism of self vs. non-self recognition and that self-targeting spacers are acquired frequently (Wei *et al.*, 2015), which implies that autoimmunity may be a significant cost.

In addition to a tradeoff / cost explanation for coexistence, outright loss of CRISPR immunity at a high enough rate could also lead to the accumulation of susceptible host. The bacterial host *Staphylococcus epidermidis* loses phenotypic functionality in its CRISPR system, either due to wholesale deletion of the relevant loci or mutation of essential sequences (i.e. the leader sequence or *cas* genes), at a rate of 10^{-4}-10^{-3} inactivation/loss events per individual per generation (Jiang *et al.*, 2013). Functional CRISPR loss has been observed in other systems as well (Garrett *et al.*, 2011; Palmer and Gilmore, 2010).

Below we replicate the serial-transfer coevolution experiments performed by Paez-Espino et al. (2013; 2015) and develop a more complex simulation-based coevolutionary model to explain the phenomenon of coexistence.

3.1 Experiments

We performed long-term daily serial transfer experiments with *Streptococcus thermophilus* and its lytic phage 2972 in milk, a model system for studying CRISPR evolution (see S4 Text for detailed methods). We measured bacteria and phage densities on a daily basis. Further, on selected days we PCR-amplified and sequenced the CRISPR1 locus, which is known to be the most active in the *S. thermophilus* host used here (Barrangou *et al.*, 2007).

From the perspective of density, phages transiently dominated the system early on, but the bacteria quickly took over and by day five appeared to be resource-limited rather than phage-limited (Fig 2a,b). This switch to host-dominance corresponded to a drop in phage populations to a density two to three orders of magnitude below that of the bacteria. Once arriving at this host-dominated state, the system either maintained quasi-stable coexistence on an extended timescale (over a month and a half), or phages continued to decline and went extinct relatively quickly (Fig 2a,b). We performed six additional replicate experiments which confirmed this dichotomy between either extended coexistence (4 lines quasi-stable for > 2 weeks) or quick phage extinction (2 lines < 1 week) (S12 Fig). In addition, a previous long-term coevolution experiment in the same system observed host-dominated coexistence for the better part of a year (Paez-Espino *et al.*, 2013; Páez-Espino *et al.*, 2015). In these experiments, phage chimerism due to a secondary contaminating phage may have extended the period of coexistence, but, even in the case where there was no contamination, host-dominated coexistence occurred for approximately one month.

Sequencing of the CRISPR1 locus revealed the rapid gain of a single spacer (albeit different spacers in different sequenced clones) followed by minor variation in spacer counts with time (S13 Fig) that is not consistent with a rapid arms race dynamic. Further, we did not observe a signature of frequent spacer loss in the CRISPR1 array.

3.2 CRISPR-phage Coevolutionary Model

While our simplified general model yielded closed form expressions for equilibria, it lacked the explicit coevolutionary dynamics of the CRISPR-phage system wherein bacteria acquire immunity via the addition of novel spacers and phages escape this

immune response via mutations. We next built a hybrid deterministic/stochastic lineage-based model similar to an earlier model by Childs et al. (2014; 2012) in which population dynamics are dictated by a set of ordinary differential equations and new equations are added to the system stochastically to simulate spacer acquisition and phage mutation. Simulation procedures are detailed in S3 Text. In addition to realistic coevolutionary dynamics, our simulations also replicate the resource dynamics of a serial dilution experiment rather than a chemostat. This final modification is essential as the two resource environments are not always comparable (e.g. Schrag and Mittler, 1996).

We model phage mutations only in the protospacer adjacent motif (PAM) region, which is the dominant location of CRISPR escape mutations (Páez-Espino *et al.*, 2015) and within which mutations eliminate the possibility of spacer re-acquisition. This approach differs from previous models which considered mutations in the protospacer region itself (e.g. Childs *et al.*, 2012; Iranzo *et al.*, 2013; Weinberger *et al.*, 2012) and thus allowed for the possibility of spacer re-acquisition. Since the probability of re-acquisition will be quite low if there are many protospacers, and since an acquisition from elsewhere in the genome that has not undergone selection for an escape mutation provides an opportunity for much more broad-based immunity, we hold that re-acquisition is the less relevant phenomenon. This difference in relevance is further compounded by the fact that as we move away from the PAM along the protospacer sequence, more substitutions are tolerated by the CRISPR matching machinery (Semenova *et al.*, 2011), meaning that mutations farther away from the PAM will be less effective at escaping immunity.

We model population dynamics using differential equations for resources:

$$\dot{R} = \frac{-evR}{z+R}\left(U + \sum_i D_i\right) \tag{5}$$

CRISPR-enabled bacteria with spacer set X_i:

$$\dot{D}_i = D_i\left(\frac{vR}{z+R} - \delta\left(\sum_j (1 - M(X_i, Y_j))P_j\right) - \alpha - \mu_L\right) \tag{6}$$

a pool of undefended bacteria with a missing or defective CRISPR system:

$$\dot{U} = U\left(\frac{vR}{z+R} - \delta\sum_i P_i\right) + \mu_L\sum_i D_i \tag{7}$$

and phages with protospacer set Y_i :

$$\dot{P}_i = \delta P_i\left(U(\beta_i - 1) + \sum_j D_j(\beta_i(1 - M(X_j, Y_i)) - 1)\right), \tag{8}$$

and stochastic events occur according to a Poisson process with rate λ:

$$\lambda = \sum_i \lambda_{B_i} + \sum_i \lambda_{P_i} + \sum_i \lambda_{K_i} \tag{9}$$

which is a sum of the total per-strain spacer-acquisition rates:

$$\lambda_{B_i} = \mu_b\delta D_i\sum_j P_j \tag{10}$$

total per-strain PAM mutation rates:

$$\lambda_{P_i} = \mu_p\beta_i\delta P_i\left(U + \sum_j(1 - M(X_i, Y_j))D_i\right) \tag{11}$$

and total per-strain PAM back mutation rates:

$$\lambda_{Q_i} = \mu_q\beta_i\delta P_i\left(U + \sum_j(1 - M(X_i, Y_j))D_i\right). \tag{12}$$

The function $M(X_i, Y_j)$ is a binary matching function between (proto)spacer content of bacterial and phage genomes that determines the presence or absence of immunity. We refer to the "order" of a host or phage strain, which is the number of evolutionary events that strain has undergone, $|X_i|$ or $n_s - |Y_i|$ respectively. The PAM back mutation rate μ_q describes the rate at which we expect a mutated PAM to revert to its original sequence (assuming the mutation is a substitution), where the back mutation rate parameter μ_q should be considerably smaller than the forward rate μ_p. While back mutation is not required to generate stable host-dominated coexistence, it greatly expands the relevant region of parameter space because it allows phages to avoid the cost we will impose on PAM mutations, discussed below, when those immune escape mutations are no longer beneficial. Recombination among viral strains could have a

similar effect by providing another route to an un-mutated or less mutated genome. Páez-Espino (2015) suggest that increased phage diversity generated via recombination can produce stable host-dominated coexistence, although we reject diversity-driven hypotheses (e.g. Childs *et al.*, 2012) based on our sequencing data.

We assume that the number of PAM mutations in a single phage genome is constrained by a tradeoff with phage fitness, as this is necessary to prevent the total clearance of protospacers from a single strain at high mutation rates. There is empirical evidence that increases in host breadth generally come at a cost for viruses due to pleiotropic effects (Ferris *et al.*, 2007), although not all individual mutations necessarily come with a cost (Duffy *et al.*, 2006). These studies, though, generally examine host range expansions to entirely novel host species rather than much more closely related CRISPR variants. That said, mutations tend to be deleterious on average (e.g. Chao, 1990) and so it is reasonable to expect PAM mutations to, on average, come with a cost. Additionally, assuming the phage in question has been associated with its host over a long evolutionary history, and that the host has possessed a CRISPR system during this time, it is reasonable to speculate that the phage has been under pressure to lose any active PAMs on its genome, and thus that the persisting PAMs may have been preserved because their loss is associated with a fitness cost. In our model, burst size presents itself as an attractive option for the incorporation of a cost, since a decrease in burst size can signify a decrease in either fecundity or viability.

The function

$$\beta_i = -\frac{c\beta_{\text{base}}}{n_s}|Y_i| + \beta_{\text{base}} \tag{13}$$

incorporates a linear cost of mutation into the phage burst size. PAM back mutation allows the phage population to recover in fitness by escaping these costs in the case where phages with relatively few mutations have already gone extinct. See Table 2 for further definitions of variables, functions, and parameters in Equations 5-13. Simulation procedures and rationale for parameter values, including phage genome size, are detailed in S3 Text.

3.2.1 Stable Host-Dominated Coexistence

Simulations with immune loss reliably produce extended coexistence within a realistic region of the parameter space (Fig 3) thus replicating our experimental results (Fig 2), and confirming our qualitative results from the simpler deterministic model (Fig 1). We observed no simulations in which autoimmunity alone produced stable coexistence. This agrees with our earlier numerical results from the general model where unrealistically high rates of autoimmunity were required to produce coexistence.

Similar to our experimental results, for a single set of parameters this model can stochastically fall into either stable coexistence or a phage-free state (Fig 3). The relative frequencies with which we see each outcome, as well as the distribution of times that phages are able to persist, depend on the specific set of parameters chosen. In particular, increasing the PAM back mutation rate will increase the probability of the coexistence outcome (Fig 4), although even in the absence of back mutation the system will occasionally achieve stable coexistence. This dependence on back mutation is caused by the combined effects of the cumulative cost we impose on PAM mutations and the inability of phages to keep up with host in a continuing arms race. In the early stages of the arms race it is optimal for phages to continue undergoing PAM mutations as the most abundant available hosts are high-order CRISPR variants, whereas once hosts are able to pull sufficiently ahead of phage in the arms race it becomes optimal for phages to feed on the lower-density but consistently available CRISPR-lacking host population (S14 Fig).

The adsorption rate, on a coarse scale, has an important effect on how the model behaves (S15 Fig). At high values of δ where we would expect phages to cause host extinction in the absence of CRISPR immunity ($\delta = 10^{-7}$) we see that long-term coexistence occurs rarely, and is negatively associated with the phage back mutation rate. In this case phages will rapidly consume the susceptible host population and crash to extinction unless they have undergone PAM mutations that lower their growth rate. The appearance of a lower order phage strain can cause a rapid decline in the susceptible host population and precipitate phage extinction. This causes a reversal in the previous trend seen with back mutation where the ability of phages to escape the costs of PAM mutation was essential to their persistence. A decrease in the adsorption rate to a very

low value ($\delta = 10^{-9}$) leads to most simulations persisting in host-dominated coexistence until the 80 day cutoff. Because both evolutionary and demographic dynamics occur much more slowly in this case, long term persistence does not necessarily imply actual stability, as suggested by our and previous (Páez-Espino *et al.*, 2015) experimental results in which coexistence eventually ends. In general, lower adsorption rates lead to longer periods of host-dominated coexistence and reduce the chance of phage extinction.

The failure of autoimmunity to produce coexistence warrants further investigation. Upon closer examination, it is clear that in the early stages of the arms race where CRISPR-enabled bacteria have not yet obtained spacers or been selected for in the host population, phages are able to proliferate to extremely high levels. During this period the CRISPR-lacking host are overwhelmed by phages and go extinct. Because autoimmunity as a mechanism of coexistence relies on the continued presence of immune-lacking host, it may not be able to function in the face of this early phage burst, which is consistently seen across all simulations where CRISPR-enabled bacteria are initiated with naive CRISPR arrays. This would further act to rule out autoimmunity as a mechanism capable of producing coexistence. There is a possibility that very low locus loss rates that reintroduce CRISPR-lacking bacteria but do not appreciably contribute to their density combined with high rates of autoimmunity could maintain high enough density susceptible host populations to sustain phage. To investigate this possibility we imposed a floor of $U > 1$ and ran another round of simulations. Even with very high rates of autoimmunity based on an upper limit of likely spacer acquisition rates ($\alpha = 50\mu_b$, $\mu_b = 10^{-5}$) the susceptible host population does not grow quickly enough to sufficiently high levels to sustain phage (S16 Fig). Thus it is not early dynamics that rule out autoimmunity but the insufficiency of the mechanism itself for maintaining large enough susceptible host populations.

3.2.2 Transient Coexistence with Low Density Phage

While we do not observe stable coexistence in any case where there is not loss of the CRISPR immune system, we did observe prolonged phage persistence in some cases where $\mu_L = \alpha = 0$ (Fig 3) and in cases with autoimmunity only ($\mu_L = 0$). Phages were able to persist at very low density ($\sim 10 - 100$ particles/mL) for as long as two months in a host-dominated setting without the presence of a CRISPR-lacking host

subpopulation (Fig 3, S17 Fig). It appears that in these cases phages are at sufficiently low density as to have a minimal effect on their host population and thus that host strain is selected against very slowly. Because the phages have undergone many PAM mutations at this point they are unable to proliferate rapidly enough between dilution events to have an easily measurable impact on the host population. Essentially, phages delay their collapse by consuming their host extremely slowly (S17 Fig). However, with an active locus loss mechanism (i.e., $\mu_L > 0$), we did not see this sustained but unstable coexistence occur, likely because the undefended hosts would have driven the phage population to higher levels and increased selection on the susceptible CRISPR variants.

4 Discussion

We paired a general model of immunity with a case study of the CRISPR immune system to characterize and contrast the potential drivers of long-term host-phage coexistence in well-mixed systems. We found that, while both a cost of immunity and the loss of immunity can lead to stable coexistence, only a loss mechanism can do so robustly within a realistic region of parameter space. We were able to reject a cost imposed on immunity (i.e., a tradeoff) as a driver of coexistence based on the lack of robustness of the equilibria produced by this mechanism. This rejection calls into question the generality of tradeoff-based explanations of host-phage coexistence, which have often been presented as the primary cause of coexistence in well-mixed systems (e.g. Bohannan and Lenski, 2000). The specific features of a given type of defense (e.g., intracellular or extracellular action) determine which mechanisms can plausibly lead to robust coexistence.

We showed that the loss of immunity can play an important role in determining coevolutionary trajectories and global population dynamics in host-parasite systems, even when only a small segment of the host population experiences these losses. Furthermore, this result, though derived from a simple, analytically tractable model, is robust to the initial conditions of the system and the addition of realistic coevolutionary dynamics. In fact, the addition of coevolutionary dynamics allowed us to reproduce additional patterns observed in our experiments, such as an early peak in the phage population and dip in the bacterial population before a transition to host dominance, as

well as stochastic switching between the possible outcomes of long term coexistence and rapid phage clearance. Our simulations reliably demonstrated a transition from an initial escalating arms race dynamic to a fluctuating selection dynamic and finally to stable predator-prey oscillations. The first of these two transitions has also been observed in previous experimental work (Hall *et al.*, 2011).

Our experiments in the *S. thermophilus* system reject a sustained arms race dynamic, since spacers did not continue to accumulate rapidly over the long term. We also reject an extended fluctuating selection dynamic based on simulations that show this type of dynamic to be unstable in the long term. Sequencing of the *S. thermophilus* CRISPR1 locus did not reveal pervasive spacer loss events. This supports our hypothesis that immune loss is at the system rather than spacer level. While our experiments do not speak to the relative importance of locus loss versus autoimmunity in the maintenance of susceptible host populations, our theoretical results reject autoimmunity as a realistic mechanism of maintenance. Our experimental setup was in serial dilution, which effectively subjects the culture to large daily perturbations in population size, ruling out any mechanism that does not produce an extremely robust coexistence regime. While autoimmunity does not lead to robust host-phage coexistence, it is a common phenomenon across organisms possessing CRISPR immune systems and may have an important effect on the evolution of host immunity in the absence of phages (Dy *et al.*, 2013; Paez-Espino *et al.*, 2013; Vercoe *et al.*, 2013; Wei *et al.*, 2015).

We note that while we explored the possibility of alternate immune tradeoff regimes (S1 Text), all of our results were derived in the context of an intracellular immune system (e.g., restriction-modification systems, CRISPR). In such systems phages are free to adsorb to defended host, and thus immunity causes phage death rather than simply preventing phage growth as in the case of extracellular resistance. Therefore, for us to observe coexistence, phages require a susceptible host population of high enough density to offset the death rate caused by immune hosts. The resulting threshold density is higher than would otherwise be needed to simply sustain phages in the absence of the adsorption-death term, which in turn increases the requisite rate of autoimmunity needed to maintain the required susceptible host population. This contributes to the finding that autoimmunity cannot account for coexistence in the systems we examine. In systems where resistance prevents phage adsorption, a

resistance-growth tradeoff in the host will likely produce a more robust coexistence regime than observed here with tradeoffs. Regardless, in either type of system a loss mechanism should reliably produce host-phage coexistence.

It is not immediately clear why bacteria would lose the functionality of their immune systems at such a high rate. Perhaps in the case of CRISPR there is some inherent instability of the locus, leading to higher rates of horizontal transfer (Garrett *et al.*, 2011; Palmer and Gilmore, 2010; Shah and Garrett, 2011). Jiang *et al.* (2013) propose that CRISPR loss is a bet-hedging strategy that allows horizontal gene transfer to occur in stressful environments (e.g., under selection for antibiotic resistance). Similarly, loss could allow escape from the cost of immunity when phages are not present, although this seems unlikely in the case of CRISPR which is up-regulated when phages are detected and relatively dormant otherwise (Agari *et al.*, 2010; Quax *et al.*, 2013; Young *et al.*, 2012), and because such a strategy would lead to poor performance in environments of frequent but transient infections. We note that a high rate of CRISPR loss and inactivation could produce a pressure for bacteria to frequently acquire new CRISPR systems through horizontal gene transfer, perhaps explaining why strains with multiple redundant CRISPR systems are frequently observed (Cai *et al.*, 2013; Horvath *et al.*, 2009).

In the case of costly and constitutive forms of defense a loss mechanism may be the only route to increased fitness in the absence of phages, perhaps driving a high loss rate in some forms of innate defense. If resistance mechanisms have a certain error rate, as seen in some cases of receptor masking or modification, then stochastic failure can also produce a susceptible host population on which phage can persist (Meyer *et al.*, 2012).

Our immune loss hypothesis predicts that the CRISPR immune system is lost at a nontrivial rate in *S. thermophilus* in addition to *S. epidermidis* (Jiang *et al.*, 2013), and possibly a range of other species. Competition experiments with phages that quantify the costs of PAM mutations, and whether or not they are cumulative, are needed to better understand the coevolutionary structure of CRISPR-phage systems in general. Other paths to sustained coexistence between CRISPR-enabled host and phages may also exist, notably anti-CRISPR phage proteins that allow phages to escape immune action similar to the methods of inactivation considered here (Bondy-Denomy *et al.*, 2015, 2013), though it is possible the presence of such proteins would simply lead to

another level of host-phage arms race.

In order to verify that the loss of defense is generally relevant to host-phage systems and not a CRISPR-specific phenomenon we must observe cases of the loss of both immunity and resistance across diverse taxa. Some examples already exist for both cases, as mentioned earlier (Jiang *et al.*, 2013; Meyer *et al.*, 2012), but in any example of host-phage coexistence it is important to consider loss of defense as a potential driver. Additionally, our work highlights that in order to invoke a tradeoff mechanism to explain the maintenance of coexistence it is necessary not only to show that a growth-immunity tradeoff exists, but that it is also sufficiently severe.

Finally, both tradeoff and loss mechanisms essentially condition the dynamics of the host-phage system on the absence of immune system functionality in some segment of the population. Our results show that the regular loss of immunity can sustain a viable phage population, leading to the maintenance of selective pressure and thus keeping immunity prevalent in the population overall. Counterintuitively, this leads us to suggest that the periodic loss of immunity drives the maintenance of a high population immune prevalence. Thus conversations about host-phage coevolution, specifically those concerning CRISPR, cannot neglect the potential susceptible individuals in a population.

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Conflict of Interest

The authors declare no conflict of interest.

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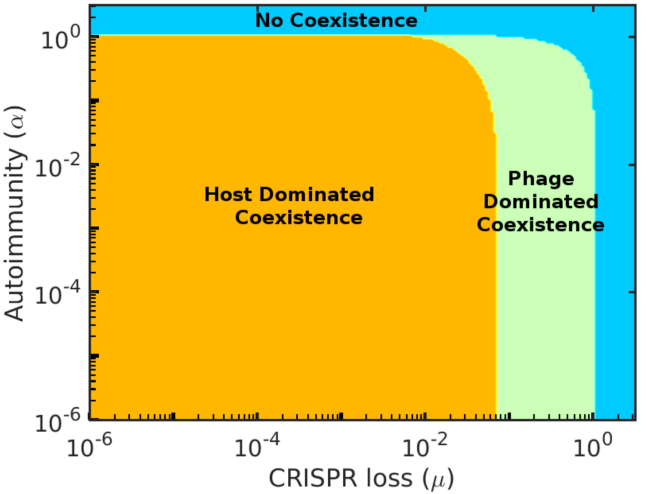
Figure legends

Figure 1: Model behavior under variations in the rates of autoimmunity (α) and CRISPR system loss (μ) Equilibria (S1 Table) derived from Equations 1-4 are shown in (a) where orange indicates a stable equilibrium with all populations coexisting and defended host dominating phage populations, green indicates that all populations coexist but phages dominate, and blue indicates that defended bacteria have gone extinct but phages and undefended bacteria coexist. In (b) we find numerical solutions to the model at 80 days using realistic initial conditions more specific to the experimental setup ($R(0) = 350$, $D(0) = 10^6$, $U(0) = 100$, $P(0) = 10^6$). In this case orange indicates coexistence at 80 days with defended host at higher density than phages, green indicates a phage-dominated coexistence at 80 days, and blue indicates that coexistence did not occur. Numerical error is apparent as noise near the orange-blue boundary. We neglect coevolution and innate immunity in this analysis ($\phi_u = 1$, $\phi_d = 0$).

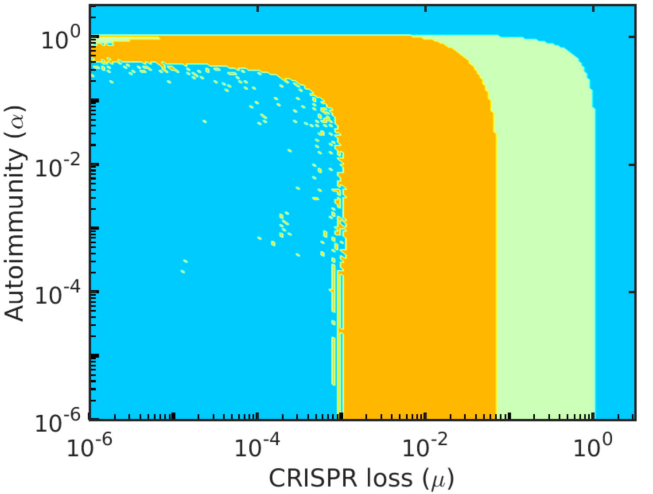
Figure 2: Serial transfer experiments carried out with *S. thermophilus* and lytic phage 2972 Bacteria are resource-limited rather than phage-limited by day five and phages can either (a) persist at relatively low density in the system on long timescales (greater than 1 month) or (b) collapse relatively quickly. These results agree with those of Paez-Espino (2015) where coexistence was observed in *S. thermophilus* and phage 2972 serially transferred culture for as long as a year. Experiments were initiated with identical starting populations and carried out following the same procedure. In (c-e) we show that our simulations replicate the qualitative patterns seen in the data, with an early phage peak, followed by host-dominated coexistence that can either be (c) stable, (d) sustained but unstable, or (e) short-lived. Each plot is a single representative simulation and simulations were ended when phages went extinct. Note that experimental data has a resolution of one time point per day, preventing conclusions about the underlying population dynamics (e.g., cycling), whereas simulations are continuous in time.

Figure 3: Distribution of phage extinction times in bacterial-dominated cultures with different possible combinations of coexistence mechanisms The peak at ≥ 75 corresponds to what we call stable coexistence (simulations ran for a maximum of 80 days). There is no significant difference between the top two panels in the number of simulations reaching the 80 day mark ($\chi^2 = 2.8904$, $df = 1$, $p - \text{value} = 0.08911$). Back mutation was set at $\mu_q = 5 \times 10^{-9}$.

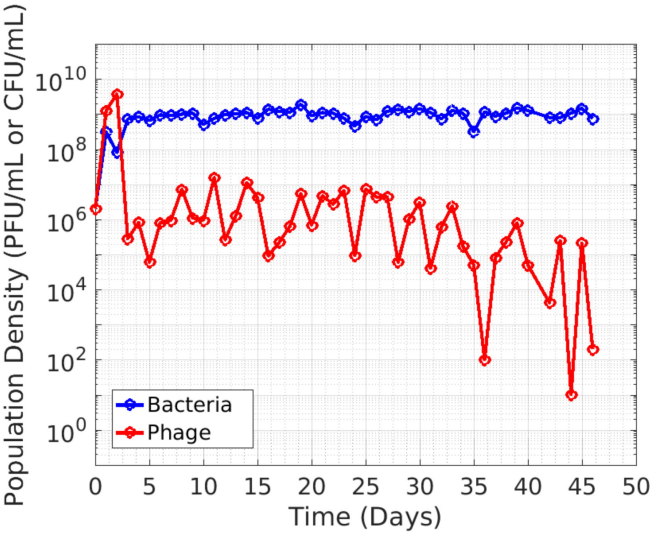
Figure 4: Distribution of phage extinction times in bacterial-dominated cultures with different rates of PAM back mutation in phages (μ_q) The peak at 80 corresponds to what we call stable coexistence (simulations ran for a maximum of 80 days). These results are shown for a locus-loss mechanism only ($\mu_L = 5 \times 10^{-4}$, $\alpha = 0$). The histogram for $\mu_q = 5 \times 10^{-8}$ is omitted as it is nearly identical to that for $\mu_q = 5 \times 10^{-9}$, indicating that the height of the coexistence peak saturates at high back mutation.


No Coexistence
Host Dominated Coexistence
Phage Dominated Coexistence
Autoimmunity (α)
CRISPR loss (μ)
10^{0}
10^{-2}
10^{-4}
10^{-6}

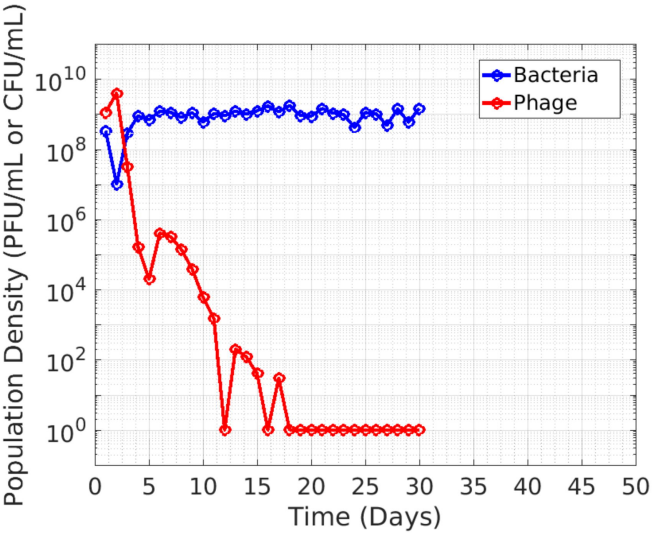
(a)


Autoimmunity (α)
CRISPR loss (μ)
10^{0}
10^{-2}
10^{-4}
10^{-6}

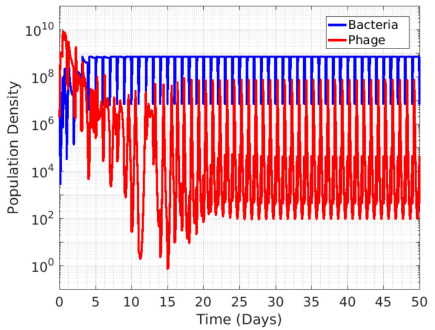
(b)


Population Density (PFU/mL or CFU/mL)
Time (Days)
Bacteria
Phage

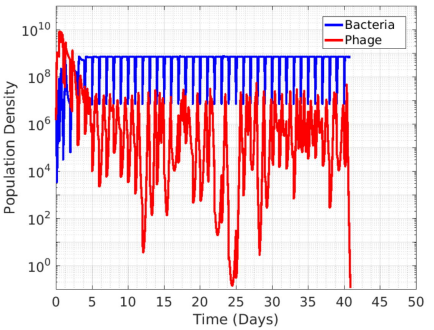
(a)


Population Density (PFU/mL or CFU/mL)
Time (Days)
Bacteria
Phage

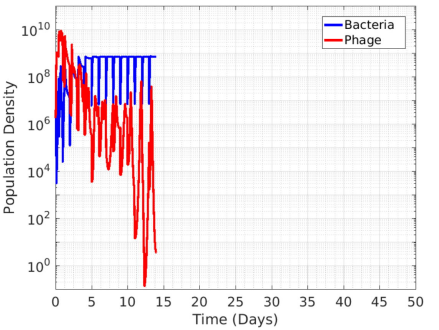
(b)


Population Density
Time (Days)
Bacteria
Phage

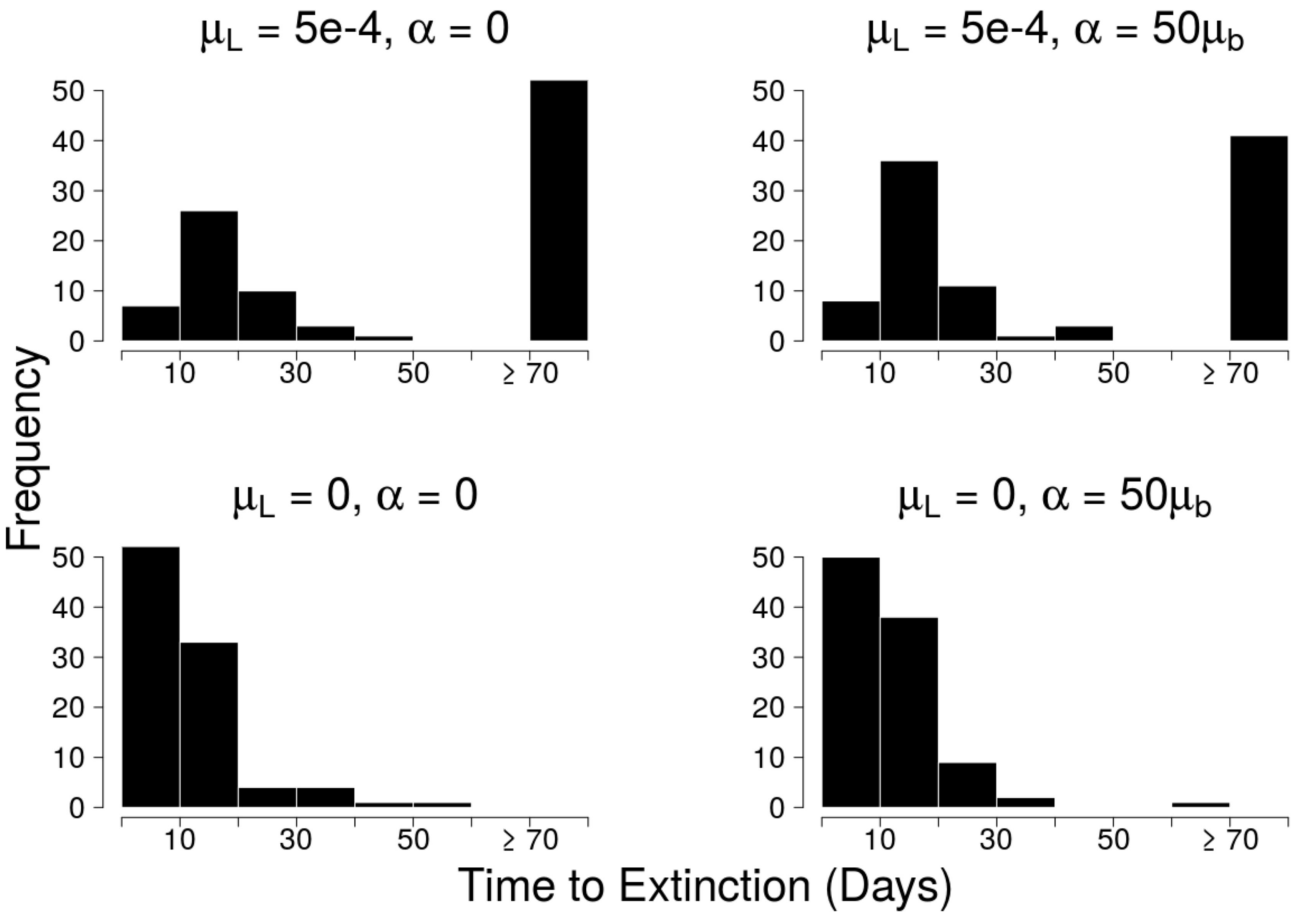
(c)

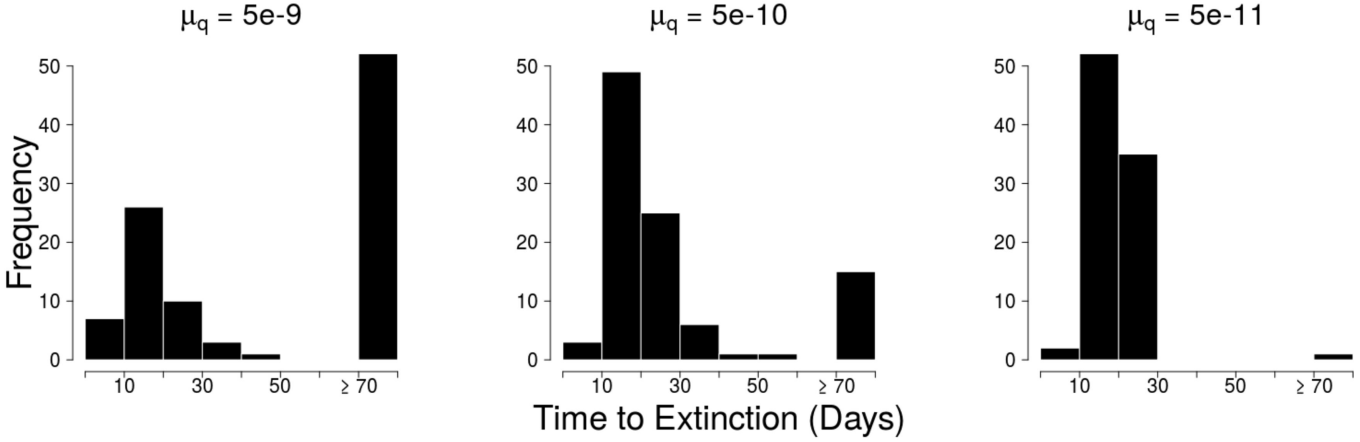

Population Density
Time (Days)
Bacteria
Phage

(d)


Population Density
Time (Days)
Bacteria
Phage

(e)


μ_L = 5e-4, α = 0
μ_L = 5e-4, α = 50μ_b
μ_L = 0, α = 0
μ_L = 0, α = 50μ_b
Frequency
0
10
20
30
40
50
10
30
50
≥ 70
Time to Extinction (Days)


μq = 5e-9
μq = 5e-10
μq = 5e-11
Frequency
0
10
20
30
40
50
10
30
50
≥ 70
Time to Extinction (Days)